Suppression of Epithelial to Mesenchymal Transitioning (EMT) Enhances Ex Vivo Reprogramming of Human Exocrine Pancreatic Tissue towards Functional Insulin Producing -Like Cells

Suppression of Epithelial-to-Mesenchymal Transitioning Enhances Ex Vivo Reprogramming of Human Exocrine Pancreatic Tissue Toward Functional Insulin-Producing β-Like Cells

Maria João Lima,[1] Kenneth R. Muir,[1] Hilary M. Docherty,[1] Robert Drummond,[2] Neil W.A. McGowan,[3] Shareen Forbes,[4] Yves Heremans,[5] Isabelle Houbracken,[5] James A. Ross,[2] Stuart J. Forbes,[6] Philippe Ravassard,[7,8] Harry Heimberg,[5] John Casey,[3] and Kevin Docherty[1]

Because of the lack of tissue available for islet transplantation, new sources of β-cells have been sought for the treatment of type 1 diabetes. The aim of this study was to determine whether the human exocrine-enriched fraction from the islet isolation procedure could be reprogrammed to provide additional islet tissue for transplantation. The exocrine-enriched cells rapidly dedifferentiated in culture and grew as a mesenchymal monolayer. Genetic lineage tracing confirmed that these mesenchymal cells arose, in part, through a process of epithelial-to-mesenchymal transitioning (EMT). A protocol was developed whereby transduction of these mesenchymal cells with adenoviruses containing Pdx1, Ngn3, MafA, and Pax4 generated a population of cells that were enriched in glucagon-secreting α-like cells. Transdifferentiation or reprogramming toward insulin-secreting β-cells was enhanced, however, when using unpassaged cells in combination with inhibition of EMT by inclusion of Rho-associated kinase (ROCK) and transforming growth factor-β1 inhibitors. Resultant cells were able to secrete insulin in response to glucose and on transplantation were able to normalize blood glucose levels in streptozotocin diabetic NOD/SCID mice. In conclusion, reprogramming of human exocrine-enriched tissue can be best achieved using fresh material under conditions whereby EMT is inhibited, rather than allowing the culture to expand as a mesenchymal monolayer. ***Diabetes*** **62:2821–2833, 2013**

From the [1]School of Medical Sciences, University of Aberdeen, Institute of Medical Sciences, Foresterhill, Aberdeen, U.K.; the [2]Medical Research Council Centre for Regenerative Medicine, Tissue Injury and Repair Group, University of Edinburgh, Chancellor's Building, Edinburgh, U.K.; the [3]Department of Surgery, University of Edinburgh, Edinburgh Royal Infirmary, Edinburgh, U.K.; the [4]Endocrinology Unit, University/British Heart Foundation Centre for Cardiovascular Science, Queen's Medical Research Institute, University of Edinburgh, U.K.; the [5]Diabetes Research Center, Vrije Universiteit Brussel, Brussels, Belgium; the [6]Medical Research Council Centre for Regenerative Medicine, Scottish Centre for Regenerative Medicine Building, University of Edinburgh, Edinburgh, U.K.; the [7]Biotechnology and Biotherapy Laboratory, CNRS UMR 7225, INSERM 975, Paris, France; and the [8]University Pierre and Marie Curie, Hôpital Pitié Salpêtrière, Paris, France.
Corresponding author: Kevin Docherty, k.docherty@abdn.ac.uk.
Received 12 September 2012 and accepted 14 April 2013.
DOI: 10.2337/db12-1256
This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db12-1256/-/DC1.
M.J.L. and K.R.M. contributed equally to this study.

Since the establishment of the Edmonton protocol, islet transplantation has become an effective and viable therapeutic option for type 1 diabetes; however, it typically requires multiple donors to achieve insulin independence (1). The lack of donor material is a significant problem and is fueling the drive toward new sources of insulin-producing cells (2). Several potential strategies exist for developing a replenishable supply of β-cells. One of these strategies is through directed differentiation of human embryonic stem cells or induced pluripotent stem cells toward a β-cell lineage, through an attempt to mimic the signaling pathways that are triggered during pancreatic development (3–13). Another strategy involves transdifferentiating or reprogramming one fully differentiated adult cell type to another (14). Thus, insulin-producing cells can be generated from liver (15–17), bone marrow (18), adipose tissue (19), and cells derived from the umbilical cord (20). Of particular relevance is the finding that murine pancreatic exocrine cells can be reprogrammed (21) in vivo and in vitro toward insulin-producing cells that are phenotypically similar to β-cells. Most of the strategies applied to murine models involved the exogenous expression of pancreatic transcription factors (TFs) that are important for normal endocrine pancreatic development (22,23). Although expression of the three transcription factors Pdx1, Ngn3, and MafA in exocrine cells of murine pancreas resulted in transdifferentiation of these cells toward the β-cell lineage in vivo (24), the same TFs were unable to generate functional β-cells in vitro (23), and further studies have shown that additional TFs such as Nkx6.1, Pax4, or IA-1 (21–23) and growth factors such as betacellulin, transforming growth factor-β (TGF-β), and epidermal growth factor (EGF) (25,26) may be important for generating functional transdifferentiated β-cells in vitro.

The successful reprogramming of murine exocrine cells has driven further studies aimed at the reprogramming of human pancreatic tissue. Implementation of the Edmonton protocol facilitated access to human cadaveric tissue that results as a byproduct of the islet isolation procedure. When placed in culture, this exocrine-enriched fraction rapidly dedifferentiates to form a mesenchymal monolayer that can be expanded through ≥20 passages (27). Several studies have attempted to expand β-cell numbers through redifferentiation of these human exocrine or islet-derived mesenchymal cells (28–32). Despite some success in

generating glucose-responsive insulin-producing cells from both islet and exocrine cell sources, the ability of the transdifferentiated cells to rescue diabetes in an animal model is still unclear.

Here, we describe how cells of the adult human exocrine pancreas obtained from the islet isolation procedure can be reprogrammed toward functional β-like cells in vitro. When placed in culture, the acinar cells undergo epithelial-to-mesenchymal transitioning (EMT), as demonstrated by genetic lineage tracing, to form a monolayer of mesenchymal cells. Efficient reprogramming was achieved using forced expression of four pancreatic TFs (Pdx1, Ngn3, Pax4, and MafA) in combination with the growth factors betacellulin and exendin-4, the vitamin nicotinamide, and small molecules that facilitate DNA binding of TFs. We show that this protocol generates predominantly glucagon-positive cells, which respond to glucose in a manner similar to that of pancreatic α-cells in vitro and in vivo. Importantly, our studies demonstrate that reprogramming of pancreatic exocrine cells toward functional insulin-producing cells could be further enhanced by suppressing EMT using inhibitors of TGF-β1 and Rho-kinase signaling pathways. The resultant cells secreted insulin in response to glucose and successfully prevented the onset of diabetes when grafted in a streptozotocin (STZ) diabetic mouse model.

RESEARCH DESIGN AND METHODS

Culture of human exocrine pancreatic fractions. All human tissue was procured with appropriate ethical consent. Human pancreata (n = 16) were isolated from brain-dead adult donors in the Scottish Islet Isolation Laboratory (SNBTS, Edinburgh, U.K.). The mean donor age was 39.4 years (range, 23–61 years) and mean BMI was 27.2 kg/m^2 (range, 22–36.5 kg/m^2). After islet isolation for clinical application, the low-purity exocrine fractions were transported to Aberdeen, where the cells were immediately plated at a density of 300,000 exocrine clusters on 75 cm^2 tissue culture flask (Greiner, Stonehouse, U.K.) and cultured in serum-containing medium prepared using RPMI 1640 (Gibco, Life Technologies, Paisley, U.K.) supplemented with 10% FBS, 10 mmol/L HEPES, 1 mmol/L sodium pyruvate (all from Gibco), and 75 μmol/L β-mercaptoethanol (Sigma Aldrich, Dorset, U.K.). Human exocrine pancreatic cells were passaged every 7 days with a solution of Trypsin (0.05%) and EDTA (0.02%; Gibco). Serum-free medium (SFM) was prepared using RPMI 1640 supplemented with 1% BSA (Sigma), 10 μg/mL insulin, and 5.5 μg/mL transferrin (both from Roche Diagnostics, West Sussex, U.K.).

Flow cytometry. Cells were incubated for 30 min with 10 μL of each primary antibody (Supplementary Table 1), washed twice with PBS, and incubated for a further 30 min with secondary antibodies (anti-mouse Ig; Silenus, Victoria, Australia). They were then washed twice in PBS and resuspended in 1% formaldehyde/PBS before analysis using a Beckman Coulter EPICS XL-MCL Flow Cytometer.

Differentiation toward adipocytes, osteoblasts, and chondrocytes. For differentiation, cells were trypsinized and resuspended in NH Adipodiff Medium, NH Osteodiff Medium, or NH Chondrodiff Medium (all from Milentyi Biotec, Surrey, U.K.), and processed as per the manufacturer's instructions.

Genetic lineage tracing. The adenoviral vectors expressing Cre recombinase under the control of the amylase promoter and the two lentiviral vectors expressing Cre recombinase under the insulin promoter and a floxed STOP dsRED promoter under control of the cytomegalovirus (CMV) promoter have been described previously (33,34). The lentiviral floxed STOP dsRED was generated by ligating dsRED into the *Bam*HI/*Xho*I–digested pTrip-CMV-Lox-STOP-Lox-EGFP-deltaU3 to replace EGFP.

Preparation of adenoviruses. Recombinant adenoviruses encoding the mouse sequences of Pdx1, MafA, Ngn3, and Pax4 (35) were prepared using the Ad-Easy system (Agilent Technologies, Edinburgh, U.K.). The adenoviruses containing *Pdx1* and *Ngn3* also expressed GFP through a downstream CMV promoter. Viral transduction was performed in SFM for 4 h at a multiplicity of infection of 100 for each virus.

Quantitative RT-PCR. Quantitative (QRT-PCR was performed as previously described (22). Data were analyzed using the $2^{-\Delta\Delta CT}$ method. Statistical analysis was performed using PRISM software and the Student t test or one-way ANOVA, followed by the Dunnet post hoc test, as appropriate. TaqMan probes are listed in Supplementary Table 3.

Immunocytochemistry and immunohistochemistry. Immunocytochemistry and immunohistochemistry were performed as previously described (13,22) using the antibodies listed in Supplementary Table 2.

C-peptide and glucagon release studies. Glucagon or C-peptide levels were measured using a human glucagon Quantikine ELISA kit (R&D Systems, Abingdon, U.K.) or a human C-peptide ELISA kit (Millipore, Livingston, U.K.).

Insulin content. Cells were harvested in Trizol reagent (Invitrogen). DNA and protein were extracted according to the manufacturer's instructions. Insulin was measured using an insulin ELISA kit (Millipore), and protein and DNA were measured as previously described (22).

Animal studies. All animal experiments were performed under U.K. Home Office regulations and with the approval of the University of Aberdeen Ethics Committee. Male 8- to 10-week-old NOD/SCID mice (NOD.CB17-Prkdcscid/NCrHsd) were obtained from Harlan Laboratories (Blackthorn, U.K.) and maintained on a 12-h light/dark cycle with ad libitum access to food. Mice were rendered diabetic by one single intraperitoneal injection of STZ (150 mg/kg), and transdifferentiated human cells were grafted under the left kidney capsule as previously described (13).

RESULTS

Human exocrine-enriched tissue dedifferentiates in culture to form a mesenchymal monolayer. The material left over from the islet isolation procedure was mainly composed of aggregates of exocrine cells, which were epithelial in nature. There was some islet debris that could be detected with dithizone or anti-insulin antibodies (Supplementary Fig. 1*A* and *B*). The amount of insulin-positive material varied between preparations but was never >2% of the total tissue. When these exocrine-enriched pancreatic fractions were placed in culture, the cells readily attached to the dish and expanded into a monolayer of proliferative cells with a morphology that resembled that of mesenchymal stromal cells (MSCs), which could be further expanded in vitro for at least 20 passages (Fig. 1*A*). This change in morphology was associated with a loss of expression of exocrine (amylase and cytokeratin 19 [CK19]), endocrine (insulin), and epithelial (E-cadherin and epithelial cell adhesion molecule) markers with a concomitant increase in the mesenchymal markers Snai2 and α-smooth muscle actin (Fig. 1*B*). Immunocytochemistry confirmed the presence of amylase-positive and CK19-positive exocrine cells as well as vimentin-positive cells on day 2, the earliest time that could be measured after plating (Fig. 2). The presence of vimentin RNA (Fig. 1*B*) probably indicated the presence of endogenous stromal cells in the exocrine fraction. The number of amylase-positive and CK19-positive cells rapidly decreased, whereas the vimentin-positive cells increased toward day 18. Interestingly, on day 4 and on day 10, there was evidence for cells that coexpressed amylase and vimentin or CK19 and vimentin, suggesting that the exocrine cells were undergoing dedifferentiation toward a mesenchymal phenotype. There also were cells that coexpressed amylase and CK19, confirming a previous report of human acinar cell plasticity (34). Flow cytometry analysis showed that >90% of the passaged cells were positive for the main characteristic mesenchymal stem cell surface markers, whereas endothelial and hematopoietic cells were very rare in these cultures (Supplementary Fig. 1*B*). In keeping with the characteristics of MSCs (36), the pancreatic-derived mesenchymal cultures were able to differentiate toward cells that stained positive for markers of adipose tissue (Oil Red O), bone (alkaline phosphatase), and cartilage (aggregan) (Supplementary Fig. 1*D*). To map the origins of the MSC population, genetic lineage tracing was undertaken using an adenovirus containing Cre-recombinase under the control of the amylase promoter and a lentiviral vector containing a CMV-driven dsRed reporter preceded by a floxed STOP cassette blocking its expression (Fig. 3*A*). In acinar cells

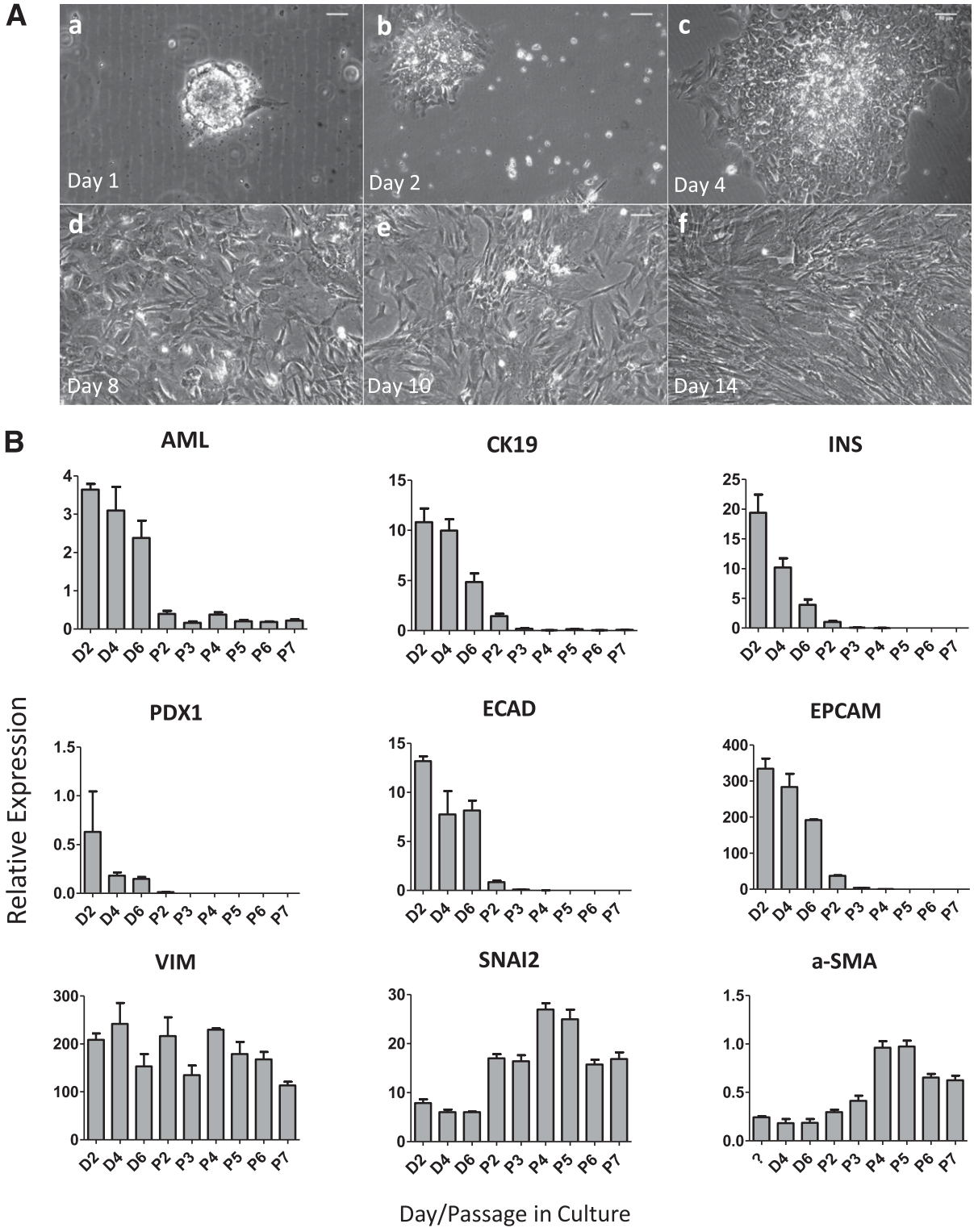

FIG. 1. Pancreatic exocrine fractions dedifferentiate toward mesenchymal cells in culture. *A*: Phase contrast images of exocrine fractions when cultured in tissue culture dishes over a 14-day period. Scale bar = 50 μm. *B*: QRT-PCR analysis of pancreatic, epithelial, and mesenchymal markers in cultured exocrine fractions from day 2 up to passage 7 after plating in tissue culture dishes. Data are presented as mean ± SEM. Expression levels are relative to glyceraldehyde 3-phosphate dehydrogenase (n = 3). AML, amylase; a-SMA, α-smooth muscle actin; D, day; ECAD, E-cadherin; EPCAM, epithelial cell adhesion molecule; INS, insulin; P, passage; SNAI2, snail homolog 2; VIM, vimentin.

transduced by both viruses, dsRed is permanently expressed, permitting continuous tracking of acinar cell fate even after amylase expression has stopped (34). The function of this lineage tracing system was validated using AR42J-B13 exocrine pancreatic cells (Supplementary Fig. 2*A*). After transduction of the human exocrine-enriched cells on day 1, dsRed-expressing cells were present on day 4 and time points thereafter (Fig. 3*A*). There was a massive increase in the number of dsRed-positive cells between day 4 and day 10, which was associated with a change

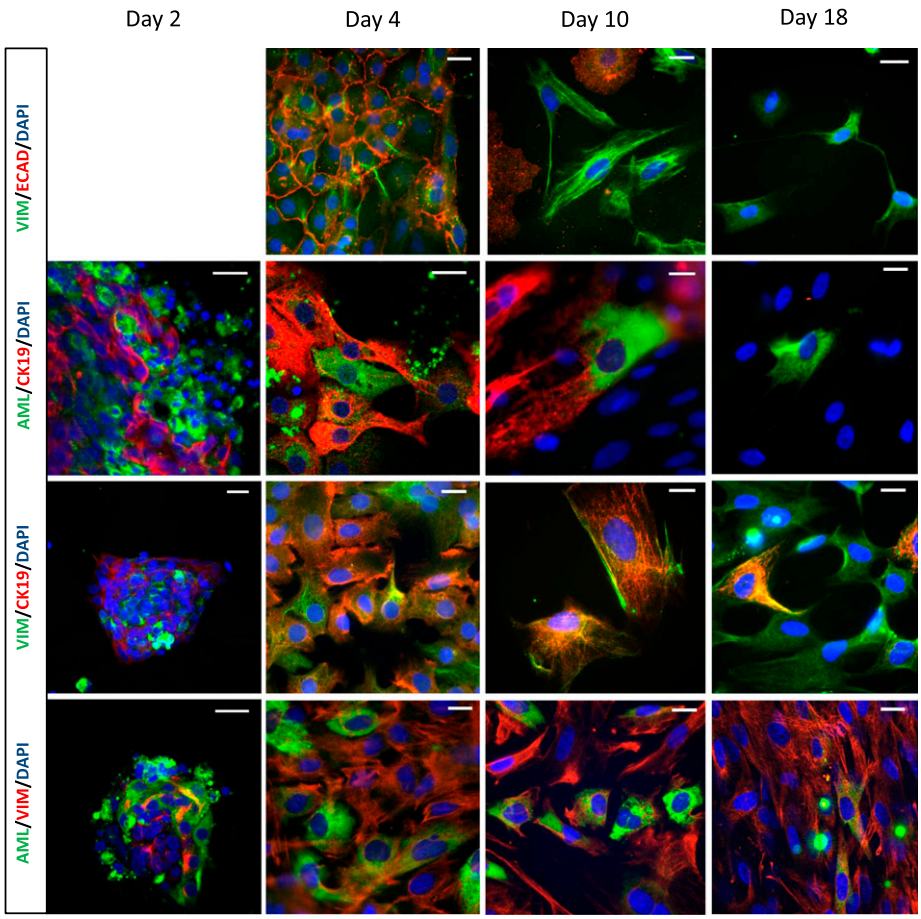

FIG. 2. Dedifferentiation of pancreatic exocrine fractions is accompanied by EMT. Immunocytochemistry of plated pancreatic exocrine fractions was performed from day 2 to day 18 in culture. The pancreatic exocrine markers amylase (AML) and CK19, the epithelial marker E-cadherin (ECAD), and the mesenchymal marker vimentin (VIM) expressions were analyzed on days 2, 4, 10, and 18 of culture. Nuclei were counterstained with DAPI. Scale bar = 20 μm.

from a rounded morphology toward a more elongated stellate shape. By day 10, the dsRed-positive cells constituted ~30% of the mesenchymal population. Immunocytochemistry showed that already by day 3 the dsRed-positive cells were positive for amylase, CK19, vimentin, and Ki67, a marker for cell proliferation (Fig. 3*C*). By day 10, the dsRed cells were all positive for vimentin and Ki67 and were negative for amylase and CK19. These results demonstrated that the vimentin-positive mesenchymal monolayer was derived by a process of EMT, although this does not rule out the presence of stromal cells within the original exocrine-enriched fractions.

The fate of β-cells also was mapped using a lentivirus containing Cre-recombinase under control of the rat insulin promoter in combination with the floxed STOP dsRed lentivirus described. The function of this system was evaluated in Min-6 β-cells (Supplementary Fig. 2*B*). Using an islet-enriched fraction, we were able to confirm the previous finding (37) that genetically labeled β-cells could transdifferentiate via a process of EMT into vimentin-positive mesenchymal cells (Supplementary Fig. 3). Therefore, it was possible that any residual islet debris (or ductal cells) in the exocrine-enriched fraction also could contribute to the MSC population that expanded in culture.

Human exocrine MSCs can be reprogrammed toward functional α-like cells. Having characterized in detail the source of the pancreatic mesenchymal monolayer, we next set out to reprogram these cells toward functional β-cells. Ectopic expression of pancreatic TFs in cultured islet-derived pancreatic MSCs previously has been accomplished with infection with adenoviral vectors (38). In the current study, a similar strategy was used to induce reprogramming of the human exocrine-derived MSCs by introducing TFs that control key stages in the developing pancreas, namely, Pdx1, Ngn3, MafA, and Pax4 (referred to collectively as the 4TFs). The individual adenoviruses transduced the pancreatic MSCs with similar efficiency as evidenced by immunocytochemistry (Supplementary Fig. 4). Individually, the TFs had no effect on the expression of glucagon, insulin, or somatostatin (Fig. 4*A*). However, in combination, the TFs significantly increased expression of glucagon with a maximal effect seen with the 4TFs (Fig. 4*A*). Under the same conditions the TFs, either individually or in combination, had no effect on the extremely low levels of expression of insulin or somatostatin. Immunocytochemistry confirmed the expression of glucagon in 40 ± 4.9% of the cells (n = 400 cells per donor, from 4 donor preparations) (Fig. 4*B*) and the absence of insulin (data not shown). A control adenovirus containing a GFP reporter had no effect on the expression of insulin, C-peptide, or any other marker (Supplementary Fig. 7*B* and *C*).

As previously described for islet-derived MSCs (28), the exocrine-derived MSCs formed cluster-like structures in SFM (Fig. 5*A*). This was accompanied by the upregulation of several pancreatic markers, including the main endocrine hormones glucagon, insulin, and somatostatin

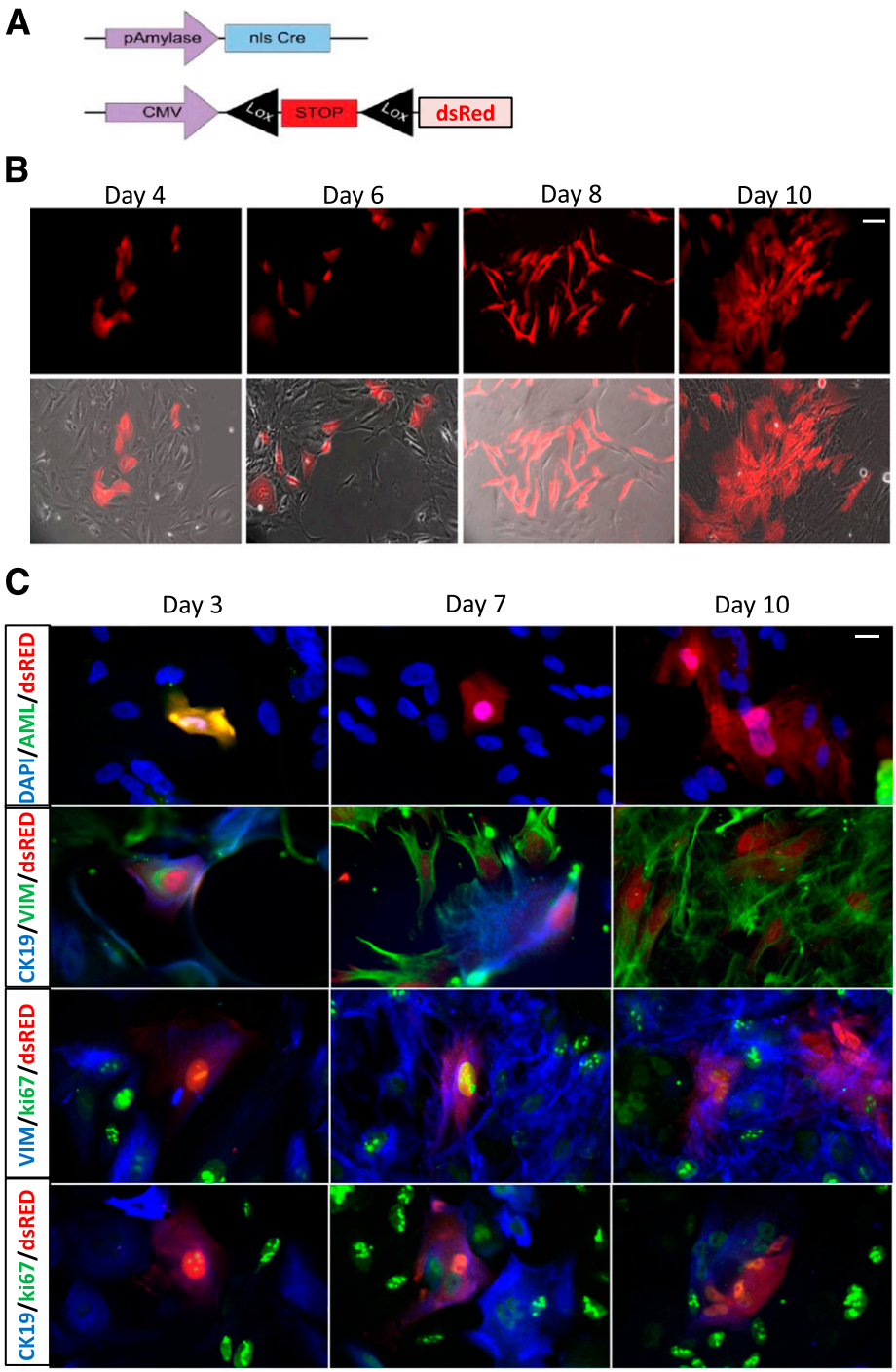

FIG. 3. Genetic lineage tracing of acinar amylase-positive cells in pancreatic exocrine fractions. *A*: Schematic representation of the two viral vectors used for tracing amylase-positive cells. *B*: The dsRed-positive cells were monitored in culture for a 10-day period. The dsRed fluorescence (*top row*) and brightfield images (*bottom row*) were analyzed at days 4, 6, 8, and 10. Scale bar = 50 μm. *C*: Immunocytochemistry was performed on days 3, 7, and 10 on traced amylase-positive cells. The exocrine pancreatic markers amylase (AML) and CK19 were analyzed along with the mesenchymal marker vimentin (VIM) and the proliferation marker ki67. Nuclei were counterstained with DAPI. Scale bar = 20 μm.

(Supplementary Fig. 5*A*), albeit at very low levels. This suggested that the action of the exogenous TFs might be further enhanced if the cells were cultured in the absence of serum. In SFM, the 4TFs were able to induce glucagon and somatostatin expression, but insulin levels were not significantly altered (Fig. 5*B*). However, in SFM, but not in serum-containing medium, glucagon, insulin, and somatostatin expression was increased by treatment with the 4TFs followed by addition of betacellulin, exendin-4, and nicotinamide (collectively referred to as BEN; Fig. 5*B*). These compounds previously have been shown to induce formation of insulin-producing cells from murine exocrine cells or adult islet cultures (22,30). Individually, these compounds had little effect (Supplementary Fig. 5*B*). Of importance was the observation that the combination of the 4TFs and BEN in SFM increased the endogenous expression levels of a number of endocrine TFs, such as Pdx1, Pax4, Nkx6.1, and Pax6 (Fig. 5*B*), suggesting that the cells had undergone transdifferentiation.

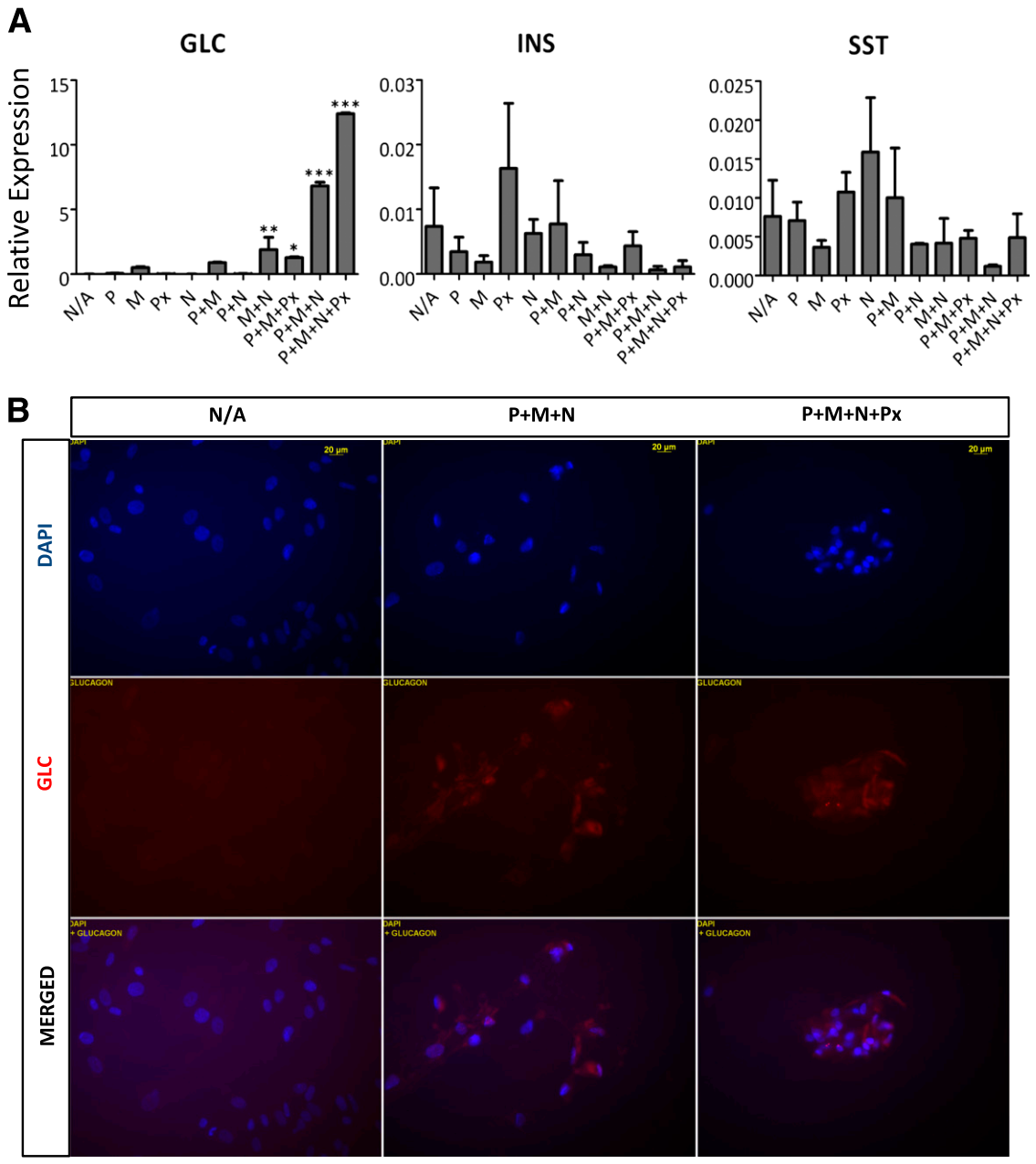

FIG. 4. Ectopic expression of pancreatic TFs reprograms exocrine pancreatic MSCs into glucagon-positive cells. *A*: Passaged human exocrine pancreatic fractions were plated in tissue culture dishes and subsequently transduced with different combinations of adenoviruses expressing the pancreatic TFs Pdx1 (P), MafA (M), Pax4 (Px), and Ngn3 (N), each with a multiplicity of infection of 100. After 7 days, QRT-PCR was performed and the data were expressed relative to glyceraldehyde 3-phosphate dehydrogenase and presented as mean ± SEM ($n = 3$). *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$. INS, insulin; GLC, glucagon; SST, somatostatin; N/A, nontreated samples. *B*: Exocrine cells treated with different combinations of adenoviruses expressing P, M, and N in the presence or absence of Px were stained for the expression of glucagon, and cell nuclei were counterstained with DAPI. Scale bar = 20 μm.

Chromatin-modifying agents enhance reprogramming. To determine the effect of modulating the chromatin structure within the MSC population before addition of the adenoviruses, the cells were incubated for 72 h with the DNA methyltransferase inhibitor 5-Aza-2′deoxycytidine and the histone deacetylase inhibitor sodium butyrate. Under these conditions, the expression of all three hormones was markedly enhanced when compared with the addition of 4TFs and BEN alone (Fig. 5*C*). It was possible to detect immunoreactive glucagon, but not insulin or C-peptide, in the media of the resultant cells. Interestingly, the secretion of glucagon was inhibited by high glucose, suggesting that the reprogrammed cells contained some of the properties of functional α-cells (Fig. 5*D*). However, immunocytochemistry demonstrated that the majority of the cells were multihormonal, expressing both glucagon and C-peptide (Fig. 5*E*). No endocrine hormones were found in nontreated exocrine-cultured cells (data not shown). Blood glucose levels were constantly higher in diabetic or nondiabetic NOD/SCID mice in which the multihormonal cells had been grafted under the kidney capsule. This is consistent with the elevated serum glucagon levels detected in fasted diabetic and nondiabetic animals (Supplementary Fig. 6).

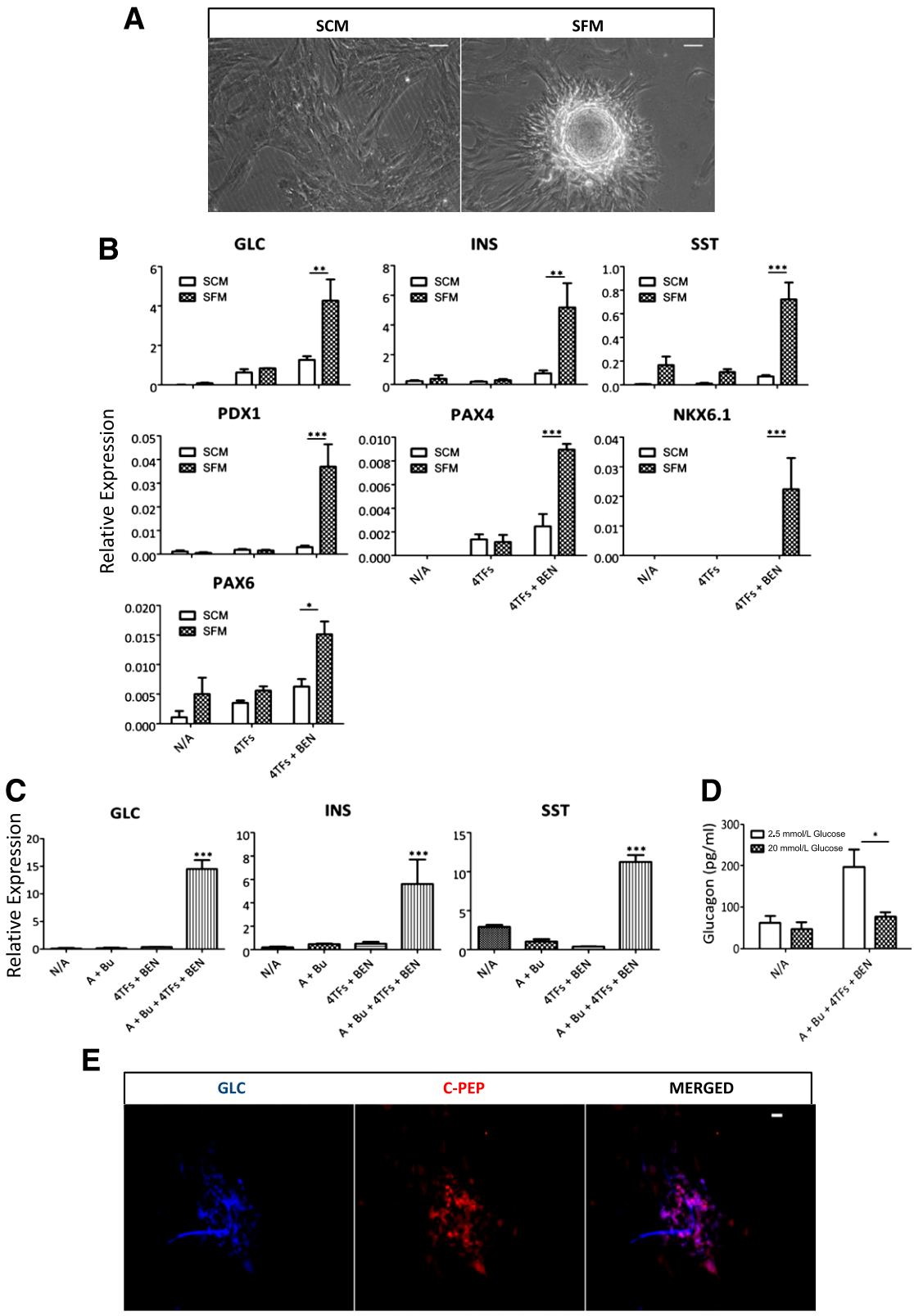

FIG. 5. SFM and chromatin-modifying reagents enhance reprogramming of exocrine pancreatic MSCs toward glucagon-producing cells. *A*: Representative phase contrast images of passaged exocrine pancreatic fractions cultured in serum-containing medium (SCM) or in SFM. Scale bar = 50 μm. *B*: Passaged exocrine pancreatic cells were cultured in SCM or SFM and transduced with adenoviruses expressing the 4TFs. After 24 h, 1 nmol/L betacellulin, 10 nmol/L exendin-4, and 10 mmol/L nicotinamide (BEN) were added to both SCM and SFM cultures. After 7 days, the cells were harvested and QRT-PCR was performed. The data are expressed relative to glyceraldehyde 3-phosphate dehydrogenase and presented as mean ± SEM (n = 3). *P < 0.05, **P < 0.01, and ***P < 0.001. *C*: Passaged exocrine pancreatic cells were cultured in SFM supplemented with 1 μmol/L 5-Aza-2′deoxycytidine (A) and/or 1 mmol/L sodium butyrate (Bu), transduced with adenoviruses (4TF), and treated with BEN as indicated. QRT-PCR was performed and the data were expressed relative to glyceraldehyde 3-phosphate dehydrogenase and presented as mean ± SEM (n = 3). ***P < 0.001 relative to nontreated samples (N/A). *D*: Glucagon secretion in culture medium of N/A or reprogrammed (A + Bu + 4TFs + BEN) exocrine cells in the presence of basal (2.5 mmol/L) or after stimulation for 1 h with high (20 mmol/L) glucose. Glucagon levels were measured by ELISA and data

Collectively, these results indicate that the treatment of pancreatic exocrine-derived MSCs with chromatin-modifying agents, followed by TFs and BEN in SFM, resulted in the formation of α-like cells that expressed glucagon along with insulin. The cells exhibited some of the functions of α-cells in an in vitro glucose release study and increased blood glucose levels when grafted into NOD/SCID mice. Reprogramming potential was specific to pancreatic exocrine-derived MSCs, because neither human skin fibroblasts nor human bone marrow MSCs responded in a manner similar to that of the reprogramming protocol (Supplementary Fig. 8). Thus, the cultured pancreatic exocrine-derived MSCs must retain some memory of their pancreatic origins.

Reversing or preventing EMT enhances reprogramming toward functional β-like cells. These experiments demonstrated that the pancreatic exocrine-derived MSCs could be induced to transdifferentiate and that optimal effects were achieved under serum-free conditions when the cells formed epithelial-like clusters. This suggested that reprogramming might be further optimized by promoting a mesenchymal-to-epithelial transition or by inhibiting the dedifferentiation of fresh exocrine-enriched epithelial tissue.

The strategy for suppressing this process of dedifferentiation involved culturing the cells in the presence of small molecules that previously have been shown to inhibit mesenchymal cell differentiation (39,40). Thus, inhibition of activin receptor–like kinases by the selective inhibitor SB431542 and the Rho-associated protein kinase inhibitor Y27632 resulted in maintenance, for a 10-day period, of the acinar and ductal cell markers amylase and CK19 at levels similar to those observed in fresh tissue (Fig. 6). In keeping with our supposition, treatment of freshly plated exocrine-enriched tissue in SFM with SB431542, Y27632, 5-Aza-2′deoxycytidine, sodium butyrate, 4TF, and BEN resulted in high levels of insulin, glucagon, and somatostatin, along with Pdx1, Pax4, MafA, and Nkx6.1, when the cells were analyzed after 10 days (Fig. 7*A*). These reprogrammed cells were glucose-responsive, releasing C-peptide in a glucose-dependent manner, and their insulin content was approximately four-fold higher than that of untreated cells (Fig. 7*B* and *C*). Clusters of cells that were highly positive for insulin and C-peptide were readily found in reprogrammed cultures (Fig. 7*D*, *a–f*), and these cells were double-positive for Pdx1 and insulin (Fig. 7*D*, *g–i*). Interestingly, some glucagon-positive cells were found in the C-peptide–positive cell clusters in a structure that closely resembles that of a mature islet (Fig. 7*D*). Both types of the reprogrammed endocrine cells generated by this protocol were monohormonal and the majority of the cells in the clusters were insulin-positive. This protocol generated an average of 18.3% (±4.9; n = 452 cells from 3 donor preparations) of insulin-producing cells in the reprogrammed cultures. No significant levels of glucagon, insulin, or other pancreatic markers were found in untreated or in exocrine cells transduced with a control Ad-GFP vector on day 10 (Supplementary Fig. 7). In summary, maintenance of the exocrine phenotype of cultured exocrine cells at the start of the treatment greatly improved the efficiency of reprogramming of exocrine pancreatic cells toward monohormonal insulin-positive cells, leading to the generation of glucose-responsive β-like cells.

The in vivo function of the reprogrammed insulin-producing cells was further determined by transplanting these cells under the kidney capsule of NOD/SCID mice that had been rendered diabetic with STZ 1 day before surgery (Fig. 8*A*). Animals that were transplanted with reprogrammed cells retained normal blood glucose levels and maintained body weight throughout the course of the experiment. Animals that were transplanted with non-reprogrammed exocrine cells, or those that were not transplanted with cells under the kidney capsule, exhibited markedly elevated blood glucose levels associated with weight loss (Fig. 8*A*). Removal of the transplanted kidney after 30 days resulted in an increase in the blood glucose levels of the animals transplanted with the reprogrammed cells (Fig. 8*A*). Human C-peptide was present only in the serum of fed mice that were transplanted with the reprogrammed insulin-producing cells (Fig. 8*B*) but was absent from the blood when fasted, suggesting that the reprogrammed cells released insulin in a glucose-responsive manner in vivo. Immunostaining of the grafted kidneys showed that the transplanted cells formed a cluster-like structure under the kidney capsule, where the center of the structure was mainly composed of strongly positive insulin-positive cells, with the majority of the glucagon-positive cells localized in the periphery of the cluster (Fig. 8*C*). The majority of the cells in this structure also were positive for the pancreatic TF Pdx1 (Fig. 8*D*). Collectively, these data support the conclusion that the exocrine pancreatic cells of the adult human pancreas can be reprogrammed toward functional insulin-producing cells. The reprogrammed cells are able to ameliorate diabetes in a diabetic mouse model and generate a cluster-like structure reminiscent of islets of Langerhans.

Finally, to determine whether the reprogramming cocktail might be mediating an effect on the proliferation of preexisting β-cells, reprogramming was performed on fresh exocrine tissue in which residual β-cells were genetically tagged with dsRed (Supplementary Fig. 9). Staining of the traced cells revealed that insulin-producing cells were not derived from preexisting β-cells, because none of the tagged dsRed-positive cells were insulin-positive after the reprogramming protocol. Even in the absence of reprogramming, insulin expression was lost in preexisting β-cells after 10 days in culture (Supplementary Fig. 9). In addition, these lineage tracing experiments further support the role of the soluble factors added in preventing EMT, because dsRed-positive cell proliferation as a mesenchymal population was observed in untreated cultures but not in cultures treated with the soluble factors alone (Supplementary Fig. 9). To investigate if residual acinar amylase, which could have a deleterious effect on transplantation, was present in the reprogrammed cells, amylase expression was investigated at the end of the reprogramming protocol (Supplementary Fig. 9*C*). As observed for residual β-cells, amylase expression disappeared in both reprogrammed and untreated cells after the 10-day protocol (Supplementary Fig. 9*C*), indicating that no traces of exocrine markers were present in the reprogrammed cultures. No evidence was found, by tracing of amylase-positive cells

represent the mean ± SEM (n = 3). *P < 0.05. *E*: Glucagon and C-peptide immunofluorescent staining in exocrine cells treated with 4TFs and BEN in the presence of 5-Aza-2′deoxycytidine and sodium butyrate. Scale bar = 20 μm. GLC, glucagon; INS, insulin; SST, somatostatin.

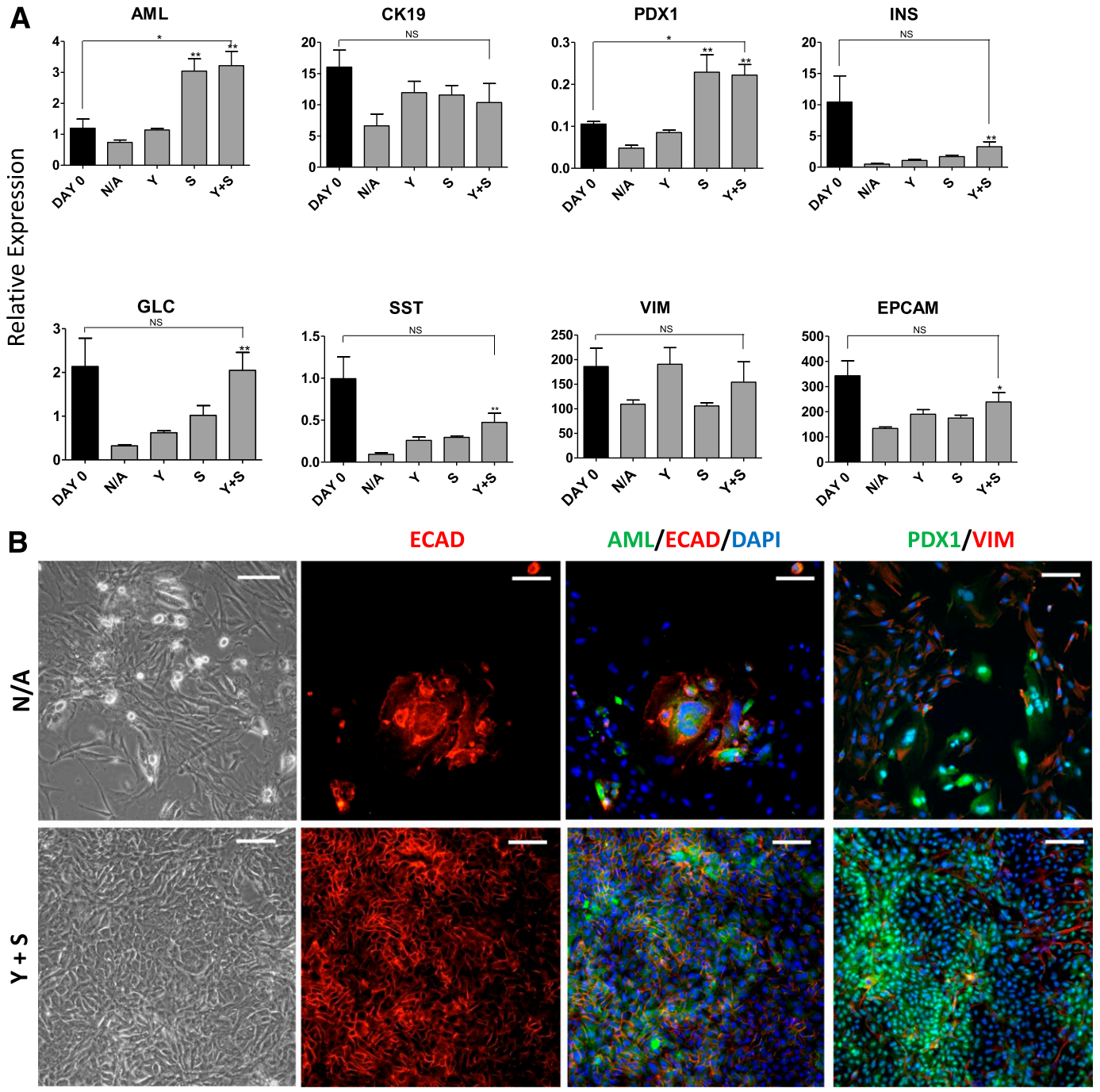

FIG. 6. The Rho-kinase and TGF-β1 pathway inhibitors suppress dedifferentiation of cultured pancreatic exocrine cells. *A*: Unpassaged exocrine pancreatic cells were plated in tissue culture dishes. After 48 h to allow attachment, cells were untreated (N/A) or treated with 2 μmol/L ρ-kinase inhibitor Y27632 (Y) and 10 μmol/L TGF-β1 inhibitor SB431542 (S) individually or in combination, and the cells were incubated for another 5 days. Treated and N/A cells as well as baseline samples after 48 h in culture (day 0) were then harvested and RNA was extracted for QRT-PCR analysis for expression of pancreatic, epithelial, and mesenchymal markers. Data are expressed relative to glyceraldehyde 3-phosphate dehydrogenase and presented as mean ± SEM (n = 3). A one-way ANOVA was performed with Dunnet post hoc test comparing treatment groups with N/A. A t test was used to compare day 2 with Y + S. For all analyses, *$P < 0.05$, **$P < 0.01$. *B*: Immunocytochemistry for the pancreatic markers amylase (AML) and Pdx1, the epithelial marker E-cadherin (ECAD), and the mesenchymal marker vimentin (VIM) in cultured exocrine pancreatic cells after 10 days in the presence of Y27632 and SB431542 (Y+S). N/A cells also were analyzed for the same markers. Nuclei were counterstained with DAPI. Scale bar = 20 μm. EPCAM, epithelial cell adhesion molecule; GLC, glucagon; INS, insulin; SST, somatostatin.

during the reprogramming protocol, to support the view that the newly generated β-like cells were derived from preexisting acinar cells (Supplementary Fig. 10). These data suggest that ductal cells are the most likely source of the new insulin-producing cells; however, lineage-tracing tools were not available to confirm this hypothesis.

DISCUSSION

In this study, we cultured exocrine cells from the fractions that are a by-product of the islet isolation procedure. Of particular importance is the fact that all of these samples were obtained from a single center and were prepared under standardized operation procedures. When placed in

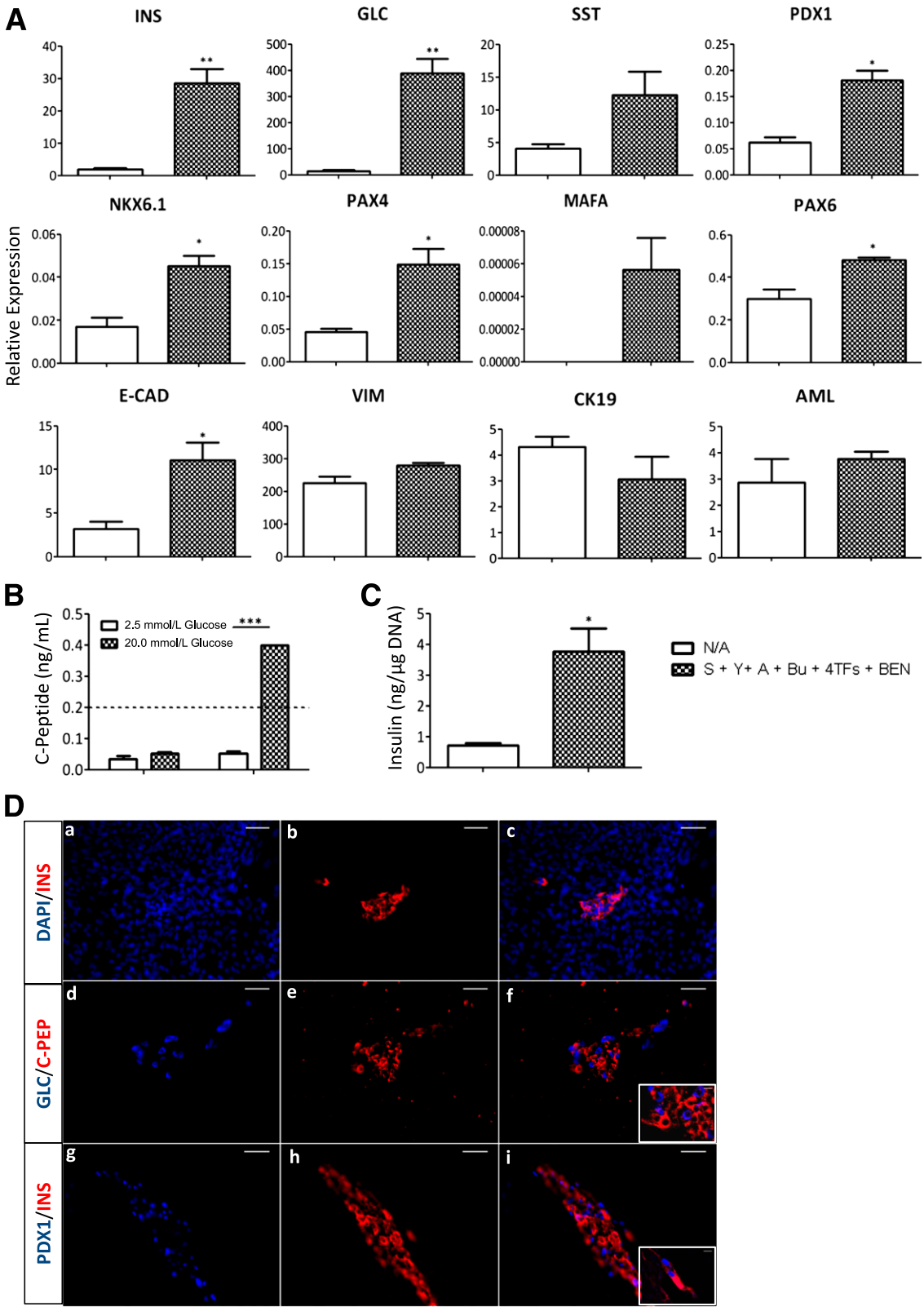

FIG. 7. The Rho-kinase and TGF-β1 pathway inhibitors enhance reprogramming toward insulin-producing cells. *A*: Unpassaged exocrine pancreatic cells were plated in tissue culture dishes. The cells were then cultured for 72 h in SFM containing Y27632 (Y), SB431542 (S), 5-Aza-2′deoxycytidine (A), and sodium butyrate (Bu). They were then transduced with adenoviruses expressing the 4TFs and cultured for 7 days in SFM containing 1 nmol/L beta-cellulin, 10 nmol/L exendin-4, and 10 mmol/L nicotinamide (BEN). Treated and untreated (N/A) cells then were harvested and RNA was extracted for QRT-PCR analysis. Data are represented as mean ± SEM and expressed relative to glyceraldehyde 3-phosphate dehydrogenase. *$P < 0.05$ or **$P < 0.01$ relative to N/A samples. *B*: Release of C-peptide to the medium in transdifferentiated (S+Y+A+Bu+4TFs+BEN) and N/A cells after incubation with 2.5 or 20 mmol/L of D-glucose for 1 h. The dashed line indicates the assay detection limit. Data are representative of triplicate experiments. ***$P < 0.001$. *C*: Insulin content of transdifferentiated (S+Y+A+Bu+4TFs+BEN) and N/A cells normalized to the DNA content of each sample. Data are representative of triplicate experiments. *$P < 0.05$. *D*: Immunostaining for insulin (INS) (*panels a–c* and *g–i*), C-peptide (C–PEP) (*panels d–f*), Pdx1 (*panels g–i*), and glucagon (GLC) (*panels d–f*) of transdifferentiated cells in culture. Nuclei were counterstained with DAPI. Data are representative of triplicate experiments. Inlets show a 2× higher magnification image of stained clusters. Scale bar = 50 μm. AML, amylase; E-CAD, E-cadherin; SST, somatostatin.

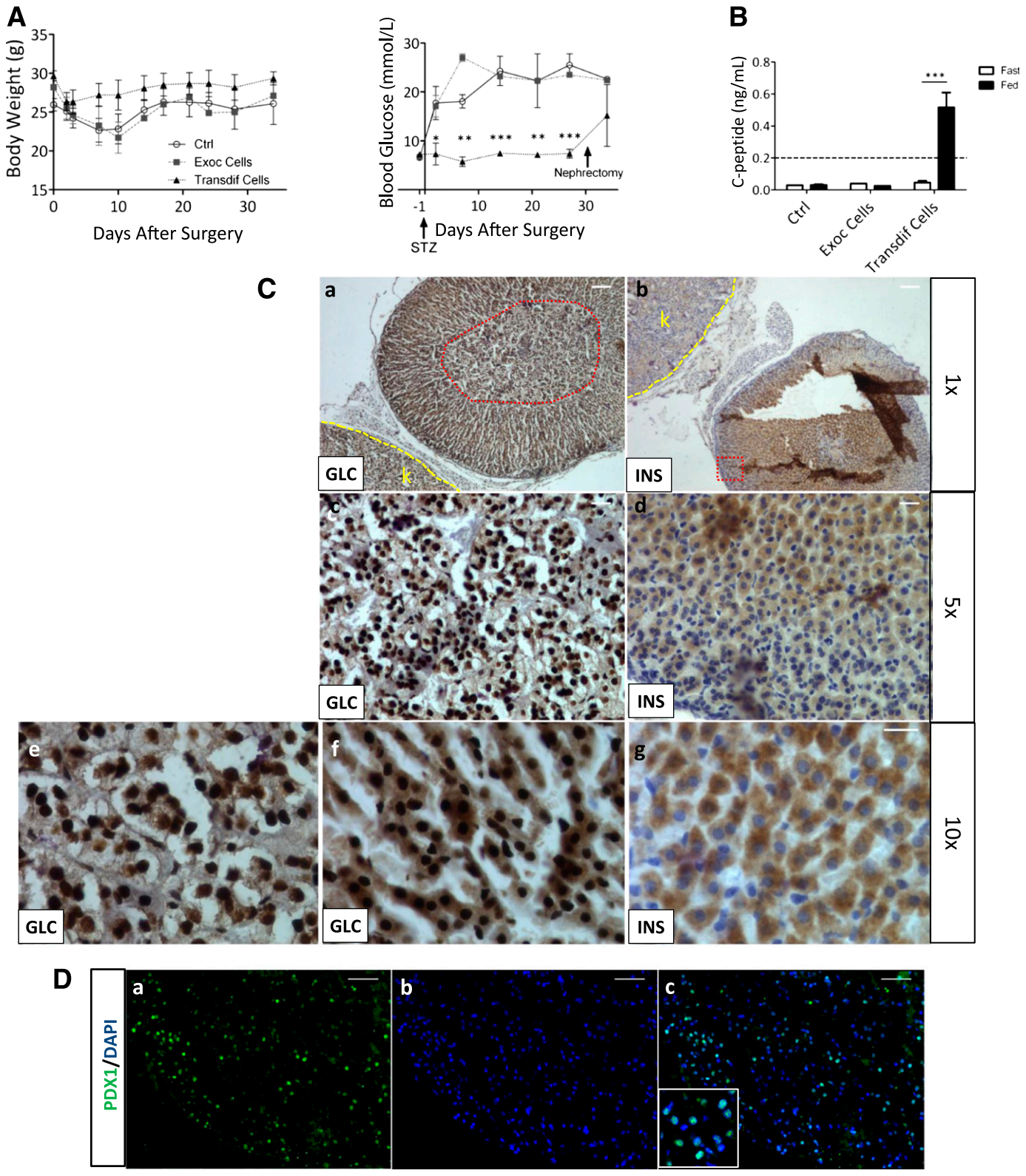

FIG. 8. Reprogrammed insulin-producing cells prevent STZ-induced diabetes in vivo. *A*: Body weight and blood glucose levels were measured in NOD/SCID mice grafted with transdifferentiated cells or exocrine pancreatic cells or in nongrafted mice (Ctrl) over a 38-day period after surgery. A single dose (150 mg/kg) of STZ was administered 1 day before surgery. Transdifferentiated cells, $n = 5$; exocrine pancreatic cells, $n = 3$; control, $n = 2$. *B*: Serum C-peptide levels were measured in NOD/SCID mice grafted with transdifferentiated cells or exocrine pancreatic cells and in nongrafted mice (Ctrl) after a 4-h starvation period (fast) or with ad libitum feeding (fed) conditions. Transdifferentiated cells, $n = 5$; exocrine pancreatic cells, $n = 3$; control, $n = 2$. *C*: Immunostaining for insulin and glucagon of grafted kidneys after kidney removal. Yellow dashed lines indicate the border between the kidney (k) and the graft. The red circle in *panel a* indicates the difference in glucagon staining observed within the cluster. A 5× higher magnification of the cells inside this circle is shown in *panel c*; 10× higher magnifications of the cells inside (*panel e*) and outside (*panel f*) the circle are shown. *Panel d* shows a 5× higher magnification of insulin staining within the area marked by the red square in *panel b*. A 10× higher magnification of insulin-positive cells present in the center of the cluster is shown in *panel g*. Scale bar for *panels a* and *b* = 100 μm. Scale bar for *panels c–g* = 20 μm. *D*: Immunofluorescent staining for Pdx1 in kidneys grafted with transdifferentiated cells. Scale bar = 50 μm. A 5× higher magnification inlet is shown. Ctrl, control; Exoc Cells, exocrine pancreatic cells; GLC, glucagon; INS, insulin; Transdif Cells, transdifferentiated cells.

culture, the exocrine pancreatic cells rapidly began to coexpress the mesenchymal marker vimentin with a quick decline in epithelial markers as the culture became uniformly single-positive for vimentin. Similar results were observed when a human islet-enriched fraction was used as starting material. Our immunocytochemistry and genetic lineage tracing results showing that cells transiently express both epithelial and mesenchymal markers clearly support the view that the human pancreatic MSC population arises from a combination of endogenous stromal cells and through EMT of all pancreatic epithelial cell types. This differs from the case of rodents, in which genetic lineage tracing studies have found no evidence for pancreatic MSCs arising via EMT from β-cells (41,42).

It previously has been shown that pancreatic MSCs derived from human isolated islets can be induced to redifferentiate when plated on extracellular matrix in response to a cocktail of growth factors under serum-free conditions (28,30,43,44), although it has been argued that this occurs only to a limited extent (45). We hypothesized that the capacity of pancreatic exocrine-derived MSCs to transdifferentiate could be enhanced by using ectopic expression of pancreatic TFs that are known to define and maintain the identity of islet cell types (46). Our approach was influenced by in vivo studies of the transdifferentiation of liver (15–17) and pancreatic exocrine cells (24) in the mouse. Our first major finding was that pretreatment of the cells with chromatin-modifying reagents, followed by adenoviruses expressing Pdx1, Ngn3, Pax4, and MafA, along with two growth factors, betacellulin and exendin 4, and the water-soluble vitamin nicotinamide could convert the pancreatic MSCs into glucagon-positive α-like cells. These multihormonal cells secreted glucagon in a physiological manner, whereby hormone release was inhibited by high-glucose concentrations in the medium. When grafted into NOD/SCID mice, these α-like cells continued to secrete glucagon in response to changes in serum glucose levels, even when the animals were rendered diabetic with streptozotocin. It was surprising that the resultant cell population was mainly composed of α-like cells because the inclusion in our reprogramming cocktail of Pax4, which favors the production of β-cells (47), was designed to tip the balance toward the β-cell lineage. During embryogenesis, α-cells and β-cells arise from a common progenitor that expresses Ngn3. There is a narrow competence window for Ngn3 expression, the length of which can markedly affect the generation of different endocrine cell types (48). In the case of the reprogrammed exocrine cells, perhaps the temporal expression of Ngn3 may dominate, and this could be addressed in further experiments by modulating expression of the exogenous Ngn3.

Our second major finding, however, was that the production of functional β-cells could be achieved by preventing dedifferentiation of the exocrine cells toward MSCs. Treatment of the exocrine-cultured cells with factors that inhibit TGF-β1 and Rho-kinase pathways (39,40), which are known to be key players in mesenchymal differentiation, prevented a decrease in exocrine marker expression in the cultures of exocrine-derived cells and the substantial expansion of pancreatic MSCs. When applied to these cells that more closely resemble the exocrine pancreas, the reprogramming protocol that previously had resulted in the formation of functional glucagon-producing cells gave rise to functional insulin-producing cells that were able to secrete insulin in response to glucose and normalize glucose levels in a streptozotocin diabetic NOD/SCID mouse model. Importantly, the levels of human C-peptide that were secreted in vitro in the presence of 20 mmol/L glucose were 0.4 ng/mL, which compares well with the amount of insulin released by equivalent numbers of cultured human islets (49).

In conclusion, we have developed a protocol that led to the successful reprogramming of human exocrine pancreatic cells toward functional β-like cells capable of preventing the onset of diabetes in a mouse diabetic model. The important finding was that fresh tissue that was predominantly epithelial could be efficiently reprogrammed toward functional β-cells, whereas expansion as a mesenchymal monolayer was less efficient, generating predominantly α-like cells. The clinical importance of these findings is that it may be possible to reprogram the exocrine pancreatic tissue that is normally discarded after the islet isolation procedure and, in a matter of weeks, provide a second batch of islets for the recipient derived from the same donor. This would obviate the need for a second donor preparation, thus increasing overall supply and also preventing additional potential donor-specific immune problems. Overall, cryopreserved exocrine tissue holds promise as a donor-recipient–specific top-up supply of islets for further use as the graft function deteriorates.

ACKNOWLEDGMENTS

This work was supported by the Wellcome Trust (085664) through the Scottish Translational Medicine and Therapeutics Initiative (K.R.M.), and by grants from the Medical Research Council (J015377/1), Grampian National Health Service Trust, and Tenovus Scotland.

No potential conflicts of interest relevant to this article were reported.

M.J.L. and K.R.M. performed experiments on the exocrine tissue, wrote the manuscript, and were involved in the planning of experiments. H.M.D. performed experiments on the exocrine tissue. R.D. undertook the fluorescence-activated cell sorter analysis and differentiation studies of MSCs. N.W.A.M. and S.F. were involved in the isolation and characterization of human islet-enriched and exocrine-enriched fractions. Y.H. and I.H. provided lentiviruses and other reagents for the reprogramming and lineage-tracing experiments and were involved with the interpretation of experiments. J.A.R. undertook the fluorescence-activated cell sorter analysis and differentiation studies of mesenchymal stromal cells. S.J.F. was involved in the planning of experiments. P.R. provided lentiviruses and other reagents for the reprogramming and lineage-tracing experiments. H.H. provided lentiviruses and other reagents for the reprogramming and lineage-tracing experiments and was involved with the interpretation of experiments. J.C. was involved in the isolation and characterization of human islet-enriched and exocrine-enriched fractions and was involved in the planning of experiments. K.D. was involved in the planning of experiments and wrote the manuscript. K.D. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

The authors thank Jan De Jonge, Vrije Universiteit Brussel, Brussels, Belgium, for technical assistance.

REFERENCES

1. Shapiro AM, Lakey JR, Ryan EA, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med 2000;343:230–238

2. Docherty K, Bernardo AS, Vallier L. Embryonic stem cell therapy for diabetes mellitus. Semin Cell Dev Biol 2007;18:827–838
3. D'Amour KA, Bang AG, Eliazer S, et al. Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. Nat Biotechnol 2006;24:1392–1401
4. Kroon E, Martinson LA, Kadoya K, et al. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nat Biotechnol 2008;26:443–452
5. Schulz TC, Young HY, Agulnick AD, et al. A scalable system for production of functional pancreatic progenitors from human embryonic stem cells. PLoS ONE 2012;7:e37004
6. Rezania A, Riedel MJ, Wideman RD, et al. Production of functional glucagon-secreting α-cells from human embryonic stem cells. Diabetes 2011;60:239–247
7. Rezania A, Bruin JE, Riedel MJ, et al. Maturation of human embryonic stem cell-derived pancreatic progenitors into functional islets capable of treating pre-existing diabetes in mice. Diabetes 2012;61:2016–2029
8. Blum B, Hrvatin SS, Schuetz C, Bonal C, Rezania A, Melton DA. Functional beta-cell maturation is marked by an increased glucose threshold and by expression of urocortin 3. Nat Biotechnol 2012;30:261–264
9. Jiang W, Shi Y, Zhao D, et al. In vitro derivation of functional insulin-producing cells from human embryonic stem cells. Cell Res 2007;17:333–344
10. Jiang J, Au M, Lu K, et al. Generation of insulin-producing islet-like clusters from human embryonic stem cells. Stem Cells 2007;25:1940–1953
11. Tateishi K, He J, Taranova O, Liang G, D'Alessio AC, Zhang Y. Generation of insulin-secreting islet-like clusters from human skin fibroblasts. J Biol Chem 2008;283:31601–31607
12. Alipio Z, Liao W, Roemer EJ, et al. Reversal of hyperglycemia in diabetic mouse models using induced-pluripotent stem (iPS)-derived pancreatic beta-like cells. Proc Natl Acad Sci USA 2010;107:13426–13431
13. Cho C-H, Hannan NR, Docherty FM, et al. Inhibition of activin/nodal signalling is necessary for pancreatic differentiation of human pluripotent stem cells. Diabetologia 2012;55:3284–3295
14. Docherty K. Reprogamming toward pancreatic beta cells. In *Stem Cell Biology and Regenerative Medicine*. Ainscough, J, Yamanaka S, Takahashi, T, Eds. New York, Humana Press, 2011, p. 177–191
15. Ferber S, Halkin A, Cohen H, et al. Pancreatic and duodenal homeobox gene 1 induces expression of insulin genes in liver and ameliorates streptozotocin-induced hyperglycemia. Nat Med 2000;6:568–572
16. Kojima H, Fujimiya M, Matsumura K, et al. NeuroD-betacellulin gene therapy induces islet neogenesis in the liver and reverses diabetes in mice. Nat Med 2003;9:596–603
17. Yechoor V, Liu V, Espiritu C, et al. Neurogenin3 is sufficient for transdetermination of hepatic progenitor cells into neo-islets in vivo but not transdifferentiation of hepatocytes. Dev Cell 2009;16:358–373
18. Karnieli O, Izhar-Prato Y, Bulvik S, Efrat S. Generation of insulin-producing cells from human bone marrow mesenchymal stem cells by genetic manipulation. Stem Cells 2007;25:2837–2844
19. Chandra V, Swetha G, Muthyala S, et al. Islet-like cell aggregates generated from human adipose tissue derived stem cells ameliorate experimental diabetes in mice. PLoS ONE 2011;6:e20615
20. Wang HS, Shyu JF, Shen WS, et al. Transplantation of insulin-producing cells derived from umbilical cord stromal mesenchymal stem cells to treat NOD mice. Cell Transplant 2011;20:455–466
21. Ogihara T, Fujitani Y, Uchida T, et al. Combined expression of transcription factors induces AR42J-B13 cells to differentiate into insulin-producing cells. Endocr J 2008;55:691–698
22. Lima MJ, Docherty HM, Chen Y, Docherty K. Efficient differentiation of AR42J cells towards insulin-producing cells using pancreatic transcription factors in combination with growth factors. Mol Cell Endocrinol 2012;358:69–80
23. Akinci E, Banga A, Greder LV, Dutton JR, Slack JM. Reprogramming of pancreatic exocrine cells towards a beta (β) cell character using Pdx1, Ngn3 and MafA. Biochem J 2012;442:539–550
24. Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA. In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. Nature 2008;455:627–632
25. Zhang T, Saunee NA, Breslin MB, Song K, Lan MS. Functional role of an islet transcription factor, INSM1/IA-1, on pancreatic acinar cell transdifferentiation. J Cell Physiol 2012;227:2470–2479
26. Baeyens L, De Breuck S, Lardon J, Mfopou JK, Rooman I, Bouwens L. In vitro generation of insulin-producing beta cells from adult exocrine pancreatic cells. Diabetologia 2005;48:49–57
27. Montgomery AM, Yebra M. The epithelial-to-mesenchymal transition of human pancreatic β-cells: inductive mechanisms and implications for the cell-based therapy of type I diabetes. Curr Diabetes Rev 2011;7:346–355
28. Gershengorn MC, Hardikar AA, Wei C, Geras-Raaka E, Marcus-Samuels B, Raaka BM. Epithelial-to-mesenchymal transition generates proliferative human islet precursor cells. Science 2004;306:2261–2264
29. Hao E, Tyrberg B, Itkin-Ansari P, et al. Beta-cell differentiation from nonendocrine epithelial cells of the adult human pancreas. Nat Med 2006;12:310–316
30. Ouziel-Yahalom L, Zalzman M, Anker-Kitai L, et al. Expansion and redifferentiation of adult human pancreatic islet cells. Biochem Biophys Res Commun 2006;341:291–298
31. Davani B, Ikonomou L, Raaka BM, et al. Human islet-derived precursor cells are mesenchymal stromal cells that differentiate and mature to hormone-expressing cells in vivo. Stem Cells 2007;25:3215–3222
32. Bar Y, Russ HA, Sintov E, Anker-Kitai L, Knoller S, Efrat S. Redifferentiation of expanded human pancreatic β-cell-derived cells by inhibition of the NOTCH pathway. J Biol Chem 2012;287:17269–17280
33. Russ HA, Bar Y, Ravassard P, Efrat S. In vitro proliferation of cells derived from adult human beta-cells revealed by cell-lineage tracing. Diabetes 2008;57:1575–1583
34. Houbracken I, de Waele E, Lardon J, et al. Lineage tracing evidence for transdifferentiation of acinar to duct cells and plasticity of human pancreas. Gastroenterology 2011;141:731–741, 741, e1–e4
35. Swales N, Martens GA, Bonné S, et al. Plasticity of adult human pancreatic duct cells by neurogenin3-mediated reprogramming. PLoS ONE 2012;7:e37055
36. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143–147
37. Russ HA, Ravassard P, Kerr-Conte J, Pattou F, Efrat S. Epithelial-mesenchymal transition in cells expanded in vitro from lineage-traced adult human pancreatic beta cells. PLoS ONE 2009;4:e6417
38. Wilson LM, Wong SH, Yu N, Geras-Raaka E, Raaka BM, Gershengorn MC. Insulin but not glucagon gene is silenced in human pancreas-derived mesenchymal stem cells. Stem Cells 2009;27:2703–2711
39. Zhang YQ, Cleary MM, Si Y, et al. Inhibition of activin signaling induces pancreatic epithelial cell expansion and diminishes terminal differentiation of pancreatic beta-cells. Diabetes 2004;53:2024–2033
40. Das S, Becker BN, Hoffmann FM, Mertz JE. Complete reversal of epithelial to mesenchymal transition requires inhibition of both ZEB expression and the Rho pathway. BMC Cell Biol 2009;10:94
41. Morton RA, Geras-Raaka E, Wilson LM, Raaka BM, Gershengorn MC. Endocrine precursor cells from mouse islets are not generated by epithelial-to-mesenchymal transition of mature beta cells. Mol Cell Endocrinol 2007;270:87–93
42. Weinberg N, Ouziel-Yahalom L, Knoller S, Efrat S, Dor Y. Lineage tracing evidence for in vitro dedifferentiation but rare proliferation of mouse pancreatic beta-cells. Diabetes 2007;56:1299–1304
43. Lechner A, Nolan AL, Blacken RA, Habener JF. Redifferentiation of insulin-secreting cells after in vitro expansion of adult human pancreatic islet tissue. Biochem Biophys Res Commun 2005;327:581–588
44. Banerjee M, Virtanen I, Palgi J, Korsgren O, Otonkoski T. Proliferation and plasticity of human beta cells on physiologically occurring laminin isoforms. Mol Cell Endocrinol 2012;355:78–86
45. Kayali AG, Flores LE, Lopez AD, et al. Limited capacity of human adult islets expanded in vitro to redifferentiate into insulin-producing beta-cells. Diabetes 2007;56:703–708
46. Bernardo AS, Hay CW, Docherty K. Pancreatic transcription factors and their role in the birth, life and survival of the pancreatic beta cell. Mol Cell Endocrinol 2008;294:1–9
47. Sosa-Pineda B, Chowdhury K, Torres M, Oliver G, Gruss P. The Pax4 gene is essential for differentiation of insulin-producing beta cells in the mammalian pancreas. Nature 1997;386:399–402
48. Johansson KA, Dursun U, Jordan N, et al. Temporal control of neurogenin3 activity in pancreas progenitors reveals competence windows for the generation of different endocrine cell types. Dev Cell 2007;12:457–465
49. Jacobs-Tulleneers-Thevissen D, Bartholomeus K, Suenens K, et al. Human islet cell implants in a nude rat model of diabetes survive better in omentum than in liver with a positive influence of beta cell number and purity. Diabetologia 2010;53:1690–1699